PRODUCTION OF BIOACTIVE FURANOSESTERTERPENE TETRONIC ACIDS AS POSSIBLE INTERNAL CHEMICAL DEFENSE MECHANISM IN THE SPONGE *IRCINIA FELIX* (PORIFERA: DEMOSPONGIAE)

SVEN ZEA, FERNANDO J. PARRA, ALEJANDRO MARTINEZ AND CARMENZA DUQUE

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Marine sponges of the genus *Ircinia* (Porifera, Demospongiae, Irciniidae) are known to produce several linear furanosesterpene tetronic acids (FTAs) with antimicrobial, cytotoxic and antitumoral properties. *Ircinia felix* is a common and abundant sponge from Santa Marta, Colombian Caribbean Sea, containing FTAs in quantities up to 4.5% of its ash-free dry tissue weight. FTA concentration was quantified by HPLC after organic extraction in individuals of *I. felix*. The following results were obtained: 1) peripheral tissues had greater concentration than internal tissues; 2) total body FTA concentration was inversely and significantly related to the ambient illumination where individuals lived (in relation to depth, and comparing locations shaded vs. open to light, and localities differing in water turbidity); 3) there was no significant variation in FTA concentration throughout the time of study (June-December 1995); 4) over a 2 month period it was found that experimental shading induced a significant increase in total body FTA concentration; 5) there was a strong FTA increase (in a scale of 1 week-2 months) when sponges were manipulated in depth transference experiments and when they were purposely injured and; 6) intact or injured individuals did not exude measurable quantities of FTAs into the surrounding medium in laboratory conditions. Together, these results indicate that FTAs have some adaptive value, but probably not in mediating external ecological interactions, but instead acting as allomonal internal suppressors and/or antibiotics. The shade-dependent production of FTAs suggest that these substances may prevent parasitisation by photosynthetic *Aphanocapsa feldmani*-type endosymbionts, when the ambient illumination is below their compensation point. Additionally, as the sponge becomes more heterotrophic under lower light levels it may have an increased need for antibiotics in the choanosome to prevent bacterial food from becoming infectious. Finally, during wound healing, increased FTA levels may also act as internal antibiotic protection. ❐ *Porifera, furanosesterterpene tetronic acids, Ircinia, chemical internal defense, Caribbean.*

Sven Zea (email szea@invemar.org.co) & Fernando J. Parra, Universidad Nacional de Colombia (Departamento de Biología), Instituto de Investigaciones Marinas y Costeras 'José Benito Vives de Andreis', INVEMAR, AA 10-16, Santa Marta, Colombia; Alejandro Martínez, Facultad de Química Farmecéutica, Universidad de Antioquia, AA 1226, Medellín, Colombia; Carmenza Duque, Departamento de Química, Universidad Nacional de Colombia, AA 100690, Bogotá, Colombia; *27 March 1999.*

The search for new drugs has led to the discovery of a variety of bioactive secondary metabolites in many terrestrial and aquatic organisms (Garson, 1994). In the marine realm, however, little is known about the use that source organisms make of these substances. It is commonly thought that sessile organisms use bioactive secondary metabolites as signals to communicate with conspecifics, to deter predators of adults (e.g. Bakus et al., 1986; Pawlik et al., 1995; Wulff, 1994; 1995), or propagules (Thompson et al., 1983), to actively or defensively compete for space (e.g. Sullivan et al., 1983; Aerts & Van Soest, 1997), or to prevent epibiosis or external damage by roaming browsers (e.g. Thompson, 1985; Walker et al., 1985; Thompson et al., 1987; Davis, 1991). Intraspecific variation in toxicity or in secondary metabolite composition has been documented in several groups of benthic sessile organisms (see review in Becerro et al., 1995). In sponges there are a few cases in which intraspecific variation of bioactive secondary metabolites, both in type and concentration, has been documented (Thompson et al., 1983, 1985, 1987; Kreuter et al., 1992; Becerro et al., 1995). This variation has been attributed to individual physiological defensive responses to differential environmental

pressures within its habitat range, especially the degree of spatial competitive interactions with neighboring macrobiota, and epibiosis prevention (Thompson et al., 1987; Becerro et al., 1995). Intra-individual (intercellular) variation in the location of bioactive secondary metabolites has been documented in a few sponges. In two cases, bioactive metabolites were found in inclusions within spherulous cells, located mostly near the surface of the sponge or around exhalant canals. These cells appear to disintegrate and release their inclusions, resulting in the exudation of metabolites through the pinacoderm, into the excurrent canals, and then into the boundary layer around the sponge. These metabolites are thought to be used for external defense-offense, but they may also be released into the mesohyl matrix for internal use (Thompson et al., 1983; Uriz et al., 1996b).

Species of *Ircinia* (Demospongiae, Dictyoceratida, Irciniidae) are known to produce linear furanosesterterpenes (Cimino et al., 1972a, b; Lumsdon et al., 1992; Urban & Capon, 1992; Capon et al., 1994; Davis & Capon, 1994; Murray et al., 1995). Recently we reported on three Caribbean species of *Ircinia* (*I. felix*, *I. strobilina* and *I. campana*), containing the novel (7E, 12E, 18R, 20Z)-variabilin as the major (58%-59.8%) furanosesterterpene tetronic acid, followed by a mixture of (8E, 13Z, 18R, 20Z)-strobilinin plus (7E, 13Z, 18R, 20Z)-felixinin (27.1%-28.6%) and a mixture of the new compounds (8Z,13Z,18R,20Z)-strobilinin and (7Z,13Z,18R, 20Z)-felixinin (13.1%-13.9%) (Martínez et al., 1995b, 1997). The greatest concentration of FTAs occur in *I. felix*, followed by *I. campana* and *I. strobilina* (Martínez, 1996). These FTAs were also found to occur as branched chain fatty acid esters, a unique combination never reported before in nature (Martínez et al., 1995a). FTAs have been demonstrated to have a variety of pharmacological properties: e.g. antibiotic (Faulkner, 1973; Martínez, 1996), cytotoxic (Martínez, 1996), antimicrobial and antitumoral (Gamboa & Pinzón, 1997), analgesic and anti-inflammatory (Del Valle & Vargas, 1997), calcium transport inhibition (Beveridge et al., 1995). Pawlik et al. (1995), argued that structurally complex secondary metabolites, which are usually present at high concentrations in sponges, can be physiologically expensive to produce, and thus must have an adaptive purpose. To test whether FTAs play an ecological role in *Ircinia* we studied the intraspecific and intra-individual variation in FTA concentration in *I. felix*, under various natural and experimental conditions. We found an inverse relationship between FTA concentration and ambient illumination, a greater concentration of FTAs in internal tissues, and an induced production of FTAs in wounded sponges. Here, we report on, and interpret these results in terms of internal defense mechanisms.

MATERIALS AND METHODS

STUDY AREA AND SOURCE ORGANISM. *Ircinia felix* (Duchassaing & Michelotti, 1864) was collected from rocky shores and mid-depth fringing reefs of Punta de Betín, and adjacent port dock-pilings in the bay of Santa Marta city (11°15'N, 74°13'W), and in rocky shores and fringing reefs of Isla Aguja, further to the NE (11°19'N, 74°12'W), Colombian Caribbean Sea. Compared to Isla Aguja, Punta de Betín generally has more turbid waters, and is subjected to greater sedimentation loads from an adjacent river, the city sewage outflows and commercial port activities. Reef corals in this locality have also suffered greater mortality, and reefs are amply colonised by sponges (Zea, 1994), especially by species of *Ircinia* (Parra, 1997). *Ircinia felix* has been described from this locality in detail by Zea (1987), and reference material is deposited in the collections of INVEMAR, Santa Marta, and the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá. In the investigated localities this species lives in densities from 4-50 individuals/40m^2; it is usually thickly encrusting to cushion-shaped, occupying areas from about 30-100cm^2, and having maximal thickness from about 1-6cm; surface is conulose, usually clean and free of epibionts, with several interspersed oscules, 3-5mmdiameter, slightly raised by a membranous collar; external color in life varies from shades of maroon and amber in specimens in well illuminated locations, to dirty cream in shaded or deep locations; internal color is cream (Parra, 1997). Below a sand-filled ectosomal reticulation (cortex), there is a layer with dense aggregations of cyanobacteria of the *Aphanocapsa feldmani*-type, whose pigments are responsible for the color of the sponge (Rützler, 1990; Vicente, 1990). This species is typical for the genus in being very tough and difficult to cut or tear largely due to a dense reinforcement of spongin fibrils throughout the mesohyl. Species of *Ircinia* also yield a characteristic sulfur-garlic stench when handled (Bergquist, 1978), releasing several sulfur and cyanide volatiles (Bonilla, 1997). Two morphotypes are readily distinguishable in Santa Marta populations of *I. felix*: 1) encrusting,

dark amber surface, oscular skin collar dark brown, and,; 2) encrusting to cushion-shaped, maroon surface, oscular skin collar white (Zea, 1987). Only the latter morphotype, which is the most abundant in the study area (Parra, 1997) was used for the chemical ecology studies presented here.

EXTRACTION AND QUANTIFICATION OF FTAs. Whole sponges or fragments were immediately frozen upon return to the field laboratory base (INVEMAR, Santa Marta). Frozen material was air-shipped to the Natural Products Laboratory of the Universidad Nacional de Colombia at Bogotá, for chemical analyses. Extraction and quantification of bioactive FTAs was developed by Martínez (1996) and Martínez et al. (1997), and standardised for this study as follows: 5-10g of wet sponge were cut and macerated first in methanol (MeOH) and then in ethyl acetate (EtOAc), each for 15mins, removing the supernatant by filtration after each solvent addition. Each supernatant was separately vacuum-dried at 35°C in a rotatory evaporator, then diluted in EtOAc, mixed, and partitioned repeatedly with H_2O to eliminate sea-water salts. The EtOAc fraction was collected, dried with anhydrous sodium sulfate, filtered, vacuum-dried and weighed. Ash-free dry weight of the solid sponge residual was obtained by subtracting from the oven-dry weight (at 115°C) the ash weight obtained after combustion in a muffle furnace at 400°C. To prolong its stability during storage, the extract was then acetylated in a 20ml mixture of acetic anhydride-pyridine (1:1). The acetylated FTAs were then purified by silica-gel column chromatography, dried and stored under nitrogen atmosphere at 0°C until use. Acetylated FTAs were subjected to HPLC for final purification using MeOH-H_2O (85:15) as mobile phase at a flow rate of 1ml min^{-1} and a Capcell Pak C_{18} (250x46mm i.d.) column as stationary phase, monitoring at 270nm. Chromatograms typically gave three peaks between 9 and 12mins, whose subfractions were known to contain five different acetylated FTAs (Martínez et al., 1997). Since the largest and latest subfraction contained pure (7E, 12E, 18R, 20Z)-variabilin acetate (henceforth refered to as variabilin), a 10µg $µl^{-1}$ solution of this compound previously obtained was used to construct a calibration curve to calculate sample concentrations. Initial quantifications were done only on variabilin, but as the structure of all FTAs were being elucidated and found to be bioactive (Martínez, 1996), data for the three peaks were pooled for further analyses and calculated as mg FTA g^{-1} ash-free dry weight of sponge.

FTA CONCENTRATION IN *IRCINIA FELIX*. *Depth and ambient illumination factors*. To initially explore if there was variation in natural variabilin concentration in tissues between individuals across various environmental conditions, two specimens of *I. felix* were collected in June 1995 at each of four depths (5, 10, 15 and 20m), in conditions of open exposure to ambient illumination at the rocky shore and fringing reef of Punta de Betín, and two more at 4-9m depth in the adjacent well-shaded pilings of the Santa Marta port dock. Statistical differences in variabilin concentration between depths and in pilings were tested by one-way ANOVA; variabilin concentration in relation to depth was investigated by regression analysis. For all statistical tests, including those mentioned below, data was tested for homogeneity of variances between treatment combinations (Bartlett test), and for normality of residuals (Kolmogorov-Smirnov test); when suitable, transformations were applied and means and standard errors back-transformed for presentation (Sokal & Rohlf, 1981).

Variabilin concentrations in peripheral vs. choanosomal tissues. To compare variabilin and total FTA concentration in peripheral tissues (including pinacoderm and peripheral choanosomal tissues a few mm below the ectosome) vs. internal tissues (deeper within the choanosome), in open vs. shaded locations, two specimens were collected at 6-7m in the Punta de Betín rocky shore in June 1995 (variabilin only), and two more at 5-6m in the adjacent dock pilings in September 1995 (variabilin and total FTA). Peripheral tissues were dissected upon return to the laboratory and stored and processed separately from choanosomal tissues. Statistical differences in variabilin and FTA concentration between tissues were compared by one-way ANOVA for each habitat separately.

Exudation. Two assays were carried out to test whether FTAs are released by undisturbed and wounded *I. felix*. 1) Nine darkened and aerated aquaria filled with 0.5L of filtered sea-water were set up in the laboratory. Six specimens of *I. felix* were carefully collected with the substratum at 10m depth in Punta de Betín in October 1995 using hammer and chisel, and each placed in an aquarium. Three specimens were deeply wounded with a razor blade whereas the other three were left undisturbed; the three aquaria

without sponges were used as controls. Unwounded specimens were checked for vitality by observing the pumping of water through oscules. After 4 hours, sponges were removed and the water stored in cold (4°C), while it was being vacuum-filtered through RP-8 cartridges to retain organics. Cartridges were then kept frozen in the dark. 2) This assay was carried out in a similar manner to the first, in November 1995, with a single specimen for each treatment. The water was immediately partitioned in EtOAc, the organic fraction dried, put under nitrogen atmosphere and stored frozen in the dark. After shipment to Bogotá, cartridges were flushed with MeOH and EtOAc to release organics and the combined extracts dried. Extracts from both experiments were acetylated and quantified as mentioned above.

Ambient- and time-related differences, and experimentally induced production. To compare total FTA concentration in localities having different environmental conditions, three specimens of *I. felix* were collected at each of two depths (10 and 20m) in Punta de Betín and Isla Aguja, in September 1995. Three more specimens were collected at each of the same depths in Punta de Betín in December 1995.

Simultaneously, shading and depth transference experiments were carried out at Punta de Betín from September to December 1995 to test for additional production of FTAs. The above-mentioned specimens served as initial and final controls for natural FTA levels. At each of the two depths, three individuals were shaded by a canopy of wire nailed to the coralline bottom and covered by a black soft polyethylene plastic. As controls, two individuals were also covered by a canopy but with transparent plastic. Also, at each depth, a PVC tube frame holding four, 5cm-wide Plexiglas beds was nailed to the bottom. Four specimens at each depth were carefully collected together with the substratum using hammer and chisel, and fixed tightly on each bed with plastic cable ties; in each frame, two specimens came from the same depth as controls for manipulative experiments, and two specimens were transferred from the reciprocal depth. All sponges were collected simultaneously in December 1995, frozen and processed.

Natural, total FTA levels for two localities, two times (initial and final), and under shading and transference treatments and controls were statistically compared against depth in a two-way ANOVA (type III sums of squares), and a Tukey multiple comparisons procedure (separately for each depth), both appropriate for unbalanced designs (SAS Institute Inc., 1988). Time changes were tested, comparing in a separated one-way ANOVA, samples from Punta de Betín collected in June (see above), September and December.

Wound-induced production. To test for FTA production in tissues after wounding, six specimens were located and tagged at 10m depth from Punta de Betín in November 1995. Initially, a wound was produced by cutting free from the edge a 2-3cm wide fragment from each specimen, which was immediately frozen to measure initial, natural FTA levels. A similarly-sized fragment was again taken, cutting parallel to the initial wound, from each of three specimens seven days later, and from each of the other three specimens 14 days later; these were frozen to measure FTA concentration changes in the wound area in the intervening period as a result of the initial wound. Initial and final FTA concentrations were compared for each time interval set by a t-student test. FTA changes for individual sponges were compared (null hypothesis of no change) by a paired t-student test, separately for each time interval.

RESULTS

NATURAL FTA LEVELS. Total body content of FTAs in *I. felix* ranged from about 1-46mg g^{-1} of ash-free dry tissue weight (Table 1; 0.1%-4.6% by weight). Significant variation in total FTA and/or variabilin (58-60% of total) content in samples was found as follows.

Ambient illumination factor. There was a significant increase in variabilin concentration in tissues of *I. felix* with increased depth at the fringing reef of Punta de Betín in June 1995 (Fig. 1). This trend followed a potential regression model [variabilin] = $0.0453\ Z^{1.82}$, R^2=0.95, $0.01<P<0.025$ (see Martínez, 1996). Since light intensity decreases exponentially with depth in sea-water, ambient illumination (not measured) was assumed to at least relate partly to variabilin concentration. Deviations from regression, however, accounted for a significant part of the model (R^2=0.04, $0.025<P<0.05$), denoting that other factors apart from depth (and light) are important in determining variabilin concentration. In addition, variabilin concentrations similar to those found at 20m, were measured in sponges collected simultaneously at 4-9m in the shaded nearby pilings of Santa Marta port (Fig. 1). These values were significantly greater than those of the sponges collected at 5m in the open fringing reef.

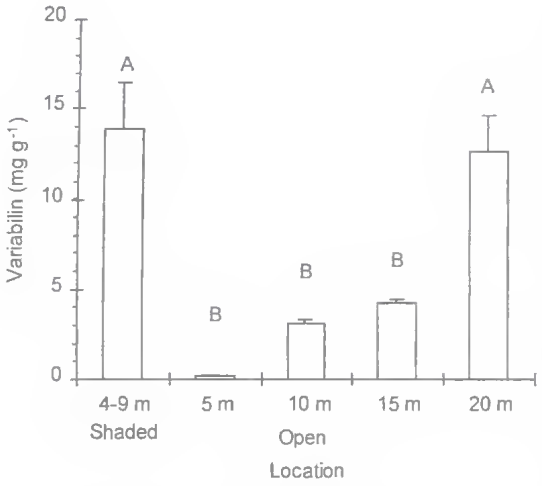

FIG. 1. Concentration of (7E,12E,18R,20Z)-variabilin (mg g^{-1} ash-free dry weight of sponge, mean±one standard error) in *Ircinia felix* under natural conditions, in shaded (commercial port dock pilings, 4-9m depth), and open (rocky-shore and fringing reef, 5-20m depth) locations at Punta de Betín in June 1995. Bars sharing the same letter are not significantly different from each other in a Tukey multiple comparison procedure (after one-way ANOVA, R^2=0.93, P=0.004, n=2 samples per location-depth).

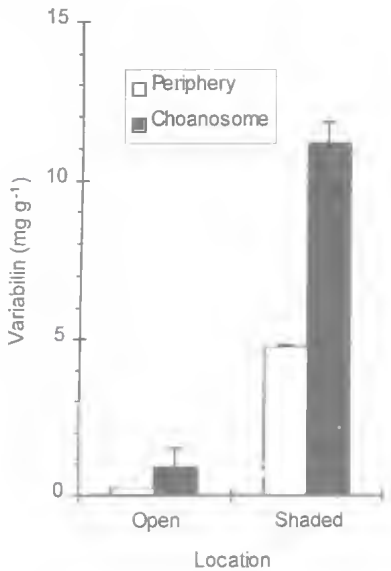

FIG. 2. Concentration of (7E,2E,18R,20Z)-variabilin (mg g^{-1} ash-free dry weight of sponge, mean±one standard error) in peripheral vs. choanosomal tissues of *Ircinia felix* in two different ambient illumination regimes (open rocky shore, 6-7m depth, June 1995; shaded dock pilings, 5m depth, Sept. 1995).

Further comparisons of total FTA concentration in September and December 1995 at Punta de Betín revealed a similar depth trend (Fig. 3A,D, representing 10 and 20m depths, respectively). The same was also true for Isla Aguja, although the absolute levels were much lower that at Punta de Betín at both depths, presumably due to greater light penetration in its generally more transparent waters. Together, these results show that ambient illumination is an important factor related to FTA natural concentration in tissues of *I. felix*.

Time factor. At 5m depth, both in open (Punta de Betín rocky shore) and shaded (dock pilings) locations, there was no significant difference in variabilin concentration between samples collected in June and September (two-way ANOVA, R^2 [time] =0.007, P= 0.48, for data, compare Fig. 1 and Fig. 2 choanosomal tissue). Similarly, for 10 and 20m depths, there were no significant differences in variabilin concentration between sponges collected in June, September and December 1995 at Punta de Betín (two-way ANOVA on time and depth, R^2 [time] =0.01, P=0.33, data from Fig. 1, and corresponding variabilin values of Fig. 3A, not shown), nor was there any difference in total FTA concentration between September and December (see Fig. 3A).

Variabilin in sponge tissues. Peripheral tissues of *I. felix* showed a significantly lower concentration of FTA than internal tissues, both in shaded (dock pilings, one-way ANOVA, R^2=0.96, P=0.0002, n=2) and open locations (Punta de Betín rocky shore, one-way ANOVA, R^2=0.99, P=0.003, n=2) at similar depths, although concentrations were significantly higher in shaded sponges in both tissues (approximately 23 times in peripheral and 12 times in choanosomal tissues to that of sponges open locations) (Fig. 2). This implies that lower FTA concentrations in peripheral tissues is not directly due to ambient illumination.

FTA PRODUCTION INDUCTION. *Exudation.* Waters surrounding individuals of *I. felix* kept in aquaria for a few hours, even when wounded, did not show traces of FTAs; both RP-8 cartridge filtration and direct EtOAc extraction gave negative results. Hence, it seems that FTAs are not released into the surrounding water of the sponge, at least in detectable quantities.

Shade-induced production. Shading experiments at 10 and 20m depths in Punta de Betín revealed a significantly greater FTA concentration when sponges were covered for 3 months with a dark

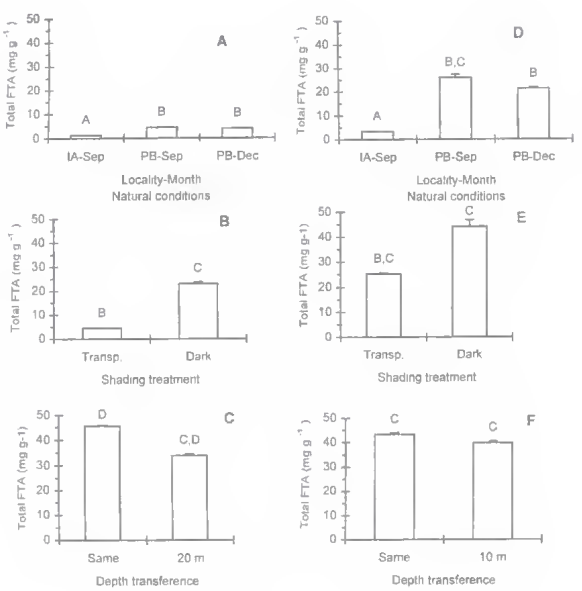

FIG. 3. Total FTA concentration (mean±one standard error, mg g^{-1} ash-free dry weight of sponge, mean±one standard error) at 10m (A-C) and 20m (D-F) depth in *Ircinia felix* under natural conditions (A and D) at Isla Aguja (IA-September 1995) and Punta de Betín (PB-September and December 1995) fringing reefs, and after shading (B and E) and depth transference experiments (C and F) at Punta de Betín. Bars on each depth having the same letter were not significantly different from each other in a Tukey multiple comparison procedure (after two-way ANOVA, Log_{10} transformed data, means and standard errors backtransformed, R^2 full model =0.99, P=0.0001, n=3 for each treatment-depth combination except for shading transparent (Transp.) controls and depth transference, in which n = 2).

shade, than when sponges were covered with transparent plastic, or were left alone under natural light. Initial and final natural levels, as well as transparent cover controls, were similar in FTA concentration at both depths, while the dark shade elicited a 5 times increase at 10m, and an about 2 times increase at 20m depth (Fig. 3B, E).

In contrast, further experiments failed to demonstrate an expected decrease in FTA concentration when sponges were transferred from 20m to 10m depth, due to a manipulation effect. Both control and reciprocal transference resulted in a significant increase after three months to a similar FTA concentration, when compared to untreated controls, regardless of depth (Fig. 3C, F).

Wound-induced production. A significant increase in FTA concentration from the initial, natural levels were found seven days (x 6 times, t-student test t=4.74, P=0.04, n=3) and 14 days (x 9 times, t=18.19, P=0.003, n=3) after wounding sponges at 10m depth in Punta de Betín fringing reef (Fig. 4). Initial levels were similar for the two sets of three sponges (t-student test, t=1.08, P=0.34). Throughout the experiment, the wound area was bleached, and a substantial release of mucus was noted while the wound was sealed.

Transference of depth experiments (see above, Fig. 3C, F) elicited FTA increases due to manipulation, probably also as a result of internal and external damaged done to sponges by squeezing them tight to the experimental frame. A few days after transference, partial bleaching and discoloration was noted; a few specimens were necrotic in areas contact to the cable ties. Slight differences between transference sets were probably due to the degree of manipulation, which elicited more-or-less damage.

DISCUSSION

This work is the first demonstration of intraspecific and between-tissue variation in FTA contents in the marine sponge *Ircinia felix*, in relation to several environmental factors and biological stress conditions. A slight degree of variation in deterrence of feeding by fish was found in pelletised extracts from Caribbean *Ircinia* species (including *I. felix*), indicating indirectly that toxic secondary metabolites may vary intraspecifically (Pawlik et al., 1995). Our results show that FTAs are not exuded free, in mucus, or after injuries, into the surrounding water, and that they also occur in a lower concentration in the peripheral tissues. Hence, an external defensive (epibiosis, predation) and offensive (competition for space) role cannot be directly ascribed to FTAs. This contrasts with the fact that *Ircinia* species usually have an epibiont-free surface (Parra, 1997), their pelletised crude extracts are not consumed by fish (Pawlik et al., 1995), and they have been found to be aggressive *in situ* against corals (Aerts & Van Soest, 1997), and these defensive--offensive mechanisms are thought to be chemically mediated. Perhaps FTAs are bad-tasting, or toxic if swallowed, hence the above-mentioned rejection by fish (J. Pawlik, pers. comm., 1998).

Nevertheless, other investigations carried out by us showed that *I. felix* passively and actively (upon injury) releases into the surrounding medium other noxious volatile compounds (Bonilla, 1997) which may be partly responsible for the above-mentioned ecological interactions.

Our results are also in contrast with those reported for other sponges that have so far shown environmentally induced variation of bioactive secondary metabolites, in which the higher concentrations of these metabolites have been found in the periphery of the sponge, or in cell types surrounding the internal canal system (e.g. *Aplysina fistularis, Rhopaloeides odorabile, Verongia aerophoba, Crambe crambe*; see Thompson et al., 1983, 1987; Kreuter et al., 1992; Uriz et al., 1996a; respectively). Some of these sponges contain metabolites which may be released into the milieu, either in areas of direct contact with other sessile organisms, or exuded into the water (inside the canals or into the surrounding medium), to act in various chemically-mediated interactions (Sullivan et al., 1983; Thompson et al., 1983; Thompson, 1985; Walker et al., 1985; Kreuter et al., 1992; Becerro et al., 1995).

However, similar to FTAs, the bioactive compound avarol and its derivatives, produced by the Mediterranean sponge *Dysidea avara,* are found only in the choanosome, specifically within choanocytes (Uriz et al., 1996b). In fact, the latter authors have cautioned against ascribing a natural function to avarol in lieu of the possibility of it being a by-product of extraction and manipulation procedures. In addition, there are no reports of environmentally induced variation in avarol. As for FTAs, the mild extraction and purification procedures used are not strong enough to either generate their functional groups, or, in case these molecules only occur in the sponge tissues as fatty acid esters (cf. Martínez et al., 1995a), to break their ester bond (Martínez, 1996). Hence, we argue for an adaptive, internal defense function of FTAs based on: 1) their presence in the tissues in free, bioactive form, and in relatively high concentrations, implying an energetic cost of production which should be balanced against other needs (cf. Pawlik et al., 1995); 2) natural (between individuals and between tissue areas of the body) and experimentally-induced variation in concentration, in relation to ambient illumination; and 3) injury-induced production.

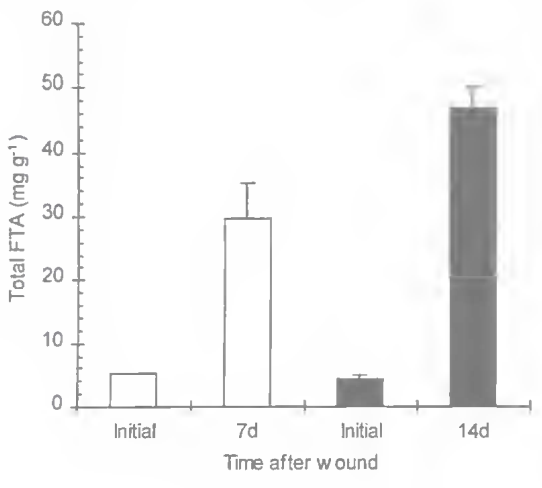

FIG. 4. Changes in total FTA concentration (mg g^{-1} ash-free dry weight of sponge, mean±one standard error) in *Ircinia felix*, 7 d (open bars) and 14 d (shaded bars) after eliciting a wound; n = 3 for each set.

One could also argue that the natural and shade-induced variations found in FTA concentration are an artifact of their lability to light, or to the result of a light-dependent biosynthetic mechanism (e.g. Kreuter et al., 1992). However, the relatively low FTA levels in choanosomal tissues in sponges located in open conditions cannot be ascribed to a light-dependent effect because the peripheral tissues have a strong pigmentation (Parra, 1997), that probably prevents light from reaching deeply into the choanosome (Wilkinson & Vacelet, 1979; Wilkinson, 1980). Similarly, finding a lower FTA concentration in peripheral vs. choanosomal tissues in discolored sponges from very dark habitats argue against light-dependent degradation.

The following are possible (and not mutually exclusive) explanations for our results in regard to internal defense.

FTA CONCENTRATION AND PRODUCTION INDUCTION IN RELATION TO AMBIENT ILLUMINATION. The causality of light (and tissue)-related FTA natural levels and shade-related production may be interpreted in the following ways.

1) FTAs are used for (partial) control of *Aphanocapsa feldmani*-type cyanobacterial symbionts. Apart from cellular mechanisms for repression (e.g. phagocytosis) of endosymbiont population growth (Simpson, 1984), antimicrobial secondary metabolites could help in their repression. Assuming an antibiotic or cytostatic affect of FTAs on *A. feldmani*, a lower

FTA concentration is retained in the ectosomal tissues of *I. felix*, where this bacteria is located, to allow it to carry out its symbiotic role. Also, an overall concentration of FTAs is maintained in inverse relation to light levels, to prevent *A. feldmani* from parasitising the sponge once its production/respiration ratio is below unity (i.e. below the compensation point). This role is difficult to test from our observations under natural conditions, since light itself may be directly responsible for *A. feldmani* control. In fact, in contrast to other, more phototrophic sponges (e.g. Seddon et al., 1992) and zooxanthellate corals (e.g. Titlyanov, 1981; Jaubert, 1981), in which symbiont density and total chlorophyll-a concentration tend to be inversely related to ambient illumination (and hence depth), in *I. felix* coloration intensity (and also chlorophyll-a concentration) decreases with decreasing illumination (unpublished results). Through cell dissociation procedures, we have failed to do precise direct counts of *A. feldmani* from *I. felix* collected at various depths and light regimes, because the dense network of spongin fibrils prevented a total separation of bacterial cells (histological procedures are needed). In short, to test this role, the sponge-cyanobacterial metabolism in relation to light, and the capacity of this cyanobacteria to live heterotrophically off the sponge, should be explored, as well as a direct, *in vitro* control of population growth by FTAs. We have succeeded in isolating the cyanobacteria, but have failed in its culture; trials are under way to test the effect of crude sponge extracts on recently isolated cyanobacteria.

2) FTAs are used to help in controlling bacterial food (e.g. Bergquist & Bedford, 1978). Assuming *I. felix* turns more heterotrophic with decreasing light (as translocation of photosynthates from cyanobacteria decreases), there may be an increased need for antibiotics in the choanosome to prevent bacterial food from parasitising the sponge. The sponge may also make use of its own stores of endosymbiotic heterotrophic bacteria as food when photosynthesis is off (Simpson, 1984; but see Wilkinson et al., 1984), and may use FTAs to regulate its bacterial flora (Thompson et al., 1983).

In the above context, the relative constancy of FTA concentration in *I. felix* throughout the study period may indicate that seasonal changes in ambient illumination and degree of phototrophy-heterotrophy are not enough to exert an important effect on the *Aphanocapsa*-FTA system. In contrast, other studied sponges have shown annual cycles of toxin production related to internal physiological cycles (e.g. reproduction), and external ecological pressures (e.g. competition for space) (Turon et al., 1996).

FTA PRODUCTION INDUCTION AS A RESULT OF INJURIES. FTAs may act as a mid-term (days to weeks) internal antibiotic protection during wound healing (e.g. after predator bites, sand scouring, etc.). However, this increase does not lead to exudation. Instead, rapid (minutes to hours) release of noxious volatile compounds (Bonilla, 1997) may act in the short term to protect wounded sponges of the genus *Ircinia*, as it has been found for other sponges (Thompson, 1985; Walker et al., 1985).

Regardless of its specific mechanism of use, it can be advanced that FTAs possess allomonal effects (cf. Whittaker & Fenny, 1977), acting as internal supressors and/or antibiotics against the sponge's own bacterial endosymbionts, or against external bacterial invaders from food or through injuries.

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